English Amended Translation of Application

TITLE OF THE INVENTION

XANTHANE AND CHITOSANE BASED POLYIONIC HYDROGELS FOR THE STABILIZATION AND CONTROLLED RELEASE OF VITAMINS

FIELD OF THE INVENTION

The present invention relates to xanthane and chitosane based hydrogels. More particularly, the present invention relates to dietary complements and to drug delivery devices where such gels are loaded with active agents such as vitamins, nucleic acids, amino acids and oligopeptides.

BACKGROUND OF THE INVENTION

The formation of chitosane or xanthane based hydrogels is known. The use of such hydrogels as an inert support for the immobilization of enzymes, or for the controlled release of certain antibiotics or anti-cancer agents is described in US patents 5,620,706 and 5,648,252. However, the application of xanthane or chitosane based hydrogels in the loading, stabilization and releasing of vitamins, nucleic acids, amino acids and oligopeptides remains to date unexplored.

The conservation of active ingredients susceptible to degradation such as vitamins, nucleic acids, amino acids and oligopeptides, constitutes one of the currant difficulties in the production of dietary complements as well as in dermatology. Exposure to light and heat accelerates this degradation.

Considering the important beneficial potential of these active ingredients, and in order to offer some protection to these active ingredients from degradation or to render them hydrophobic, several vehicles such as tablets, capsules, gel capsules, cremes, ointments, gels, aqueous dispersions (emulsions) and solutions were developed. However, several disadvantages of these synthetic preparations, such as the potential for irritation and toxicity, were alluded to.

Unable to find an adequate vehicle among the conventional excipients, the use of synthetic polycations was recently proposed. For example in European patent application 504 066 A1, 1992, filed by Oreal, France, cosmetic compositions containing a dispersion of solid active particles whose surface is covered with a cationic polymer, are suggested. However, the object of the cationic surface layer is to render the composition as a whole more stable as opposed to stabilizing a particular ingredient facing a tendency towards degradation.

There thus remains an important need to develop a new vehicle useful in food preparations, cosmetology and dermatology holding active photosensitive and thermosensitive active ingredients such as vitamins, nucleic acids, amino acids and oligopeptides. One of the objects of the present invention is to provide dietary and dermatological preparations containing a natural polyionic hydrogel allowing for the protection of photo and thermo-sensitive active ingredients, without exhibiting a potential for irritation or toxicity. Yet another object of the present invention is to provide a method allowing for the incorporation of these active ingredients into a natural polyionic hydrogel.

Other objects, advantages and features of the present invention will become more apparent upon reading the following non-restrictive description of preferred embodiments, which are exemplary and should not be interpreted as limiting the scope of the present invention. It should be understood however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

SUMMARY OF THE INVENTION

In general terms, the invention provides a thermo and photo stable composition comprised of a hydrogel composed of a xanthane and chitosane complex, wherein the hydrogel comprises at least one thermo or

photo sensitive substance selected from the group consisting of: vitamins, amino acids, nucleic acids and oligopeptides, and wherein the hydrogel is adapted to release the above mentioned thermo- or photo sensitive substances in an animal or human subject. Preferably, the thermo or photo sensitive substances are present in a proportion of 5 to 25% by weight of the total composition. More preferably, these substances are selected from vitamins A, B, C, D, E and K.

The invention also provides a process for the preparation of the inventive compositions, the process comprising the following steps:

(a) dissolving liposoluble substances in an appropriate solvent;

(b) adding this solution, while stirring, to a xanthate solution;

(c) pulverizing the mixture from step (b) to a chitosane solution;

(d) recuperating the so-formed hydrogel;

(e) incorporating by diffusion of the liposoluble substances from step (a) to the hydrogel;

all the steps are carried out essentially in the absence of oxygen and light.

In an other process, according to the present invention, the composition of the invention could be obtained by the following steps:

(a) pulverizing a xanthane solution in a chitosane solution;

(b) recovering the so-formed hydrogel will be characterized by lyophilizing the hydrogel;

(c) introducing the lyophilized hydrogel to an aqueous solution comprising the hydrosoluble substances that can be advantageously stabilized by the addition of amino acids such as L-cysteine, L-cystine and L-methionine or a mixture of the latter or by the addition of tri-peptides;

(d) incorporating the hydrosoluble substances in the hydrogel by diffusion of the hydrosoluble substances during the swelling of the hydrogel, the said hydrogel having a degree of swelling of 2000% or more.

In accordance with the present invention there is also provided a method of use for these hydrogels in dermatology or as a dietary complement.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Complexation between the chitosane and xanthane

Figure 2 Assembly scheme for the kinetic study

Figure 3 Amount of Vitamin C liberated in function of time
Coded sample VS2L. Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 1800%

Figure 4 Rate of release of vitamin C in function of time.
Coded sample VS2L

Figure 5 Weight of vitamin C in fonction of time
Coded sample VS2R. Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 2200%

Figure 6 Rate of release of vitamin C in function of time.
Coded sample VS2R

Figure 7 Variation of the percentage of vitamin C released in function of time
Sample VS2M Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 3500%

DETAILED DESCRIPTION OF THE INVENTION

The present detailed description reveals dermatological and dietary compositions comprising one or more active ingredients, photo or thermo sensitive to degradation. These compositions comprising a polyionic hydrogel, generated from a chitosane and xanthane complex, able to incorporate and protect the photo and thermo sensitive active

ingredients, as well as to improve their activity through a controlled release effect.

Two processes for the incorporation of the active ingredients into the chitosane and xanthane hydrogel are also described. The liposoluble active ingredients are incorporated into the hydrogel by a first method, in the course of the formation of the hydrogel. The hydrosoluble active ingredients are incorporated into the hydrogel, by a diffusion method, following the formation of the hydrogel.

The present invention also demonstrates, in a surprising and innovating manner, the possibility of incorporating various vitamins or other ingredients such as amino acids, nucleic acids and oligopeptides into a hydrogel and to then release these substances via different paths such as oral dosages, suppositories, cremes, ointments, gels, solutions, transcutaneous devices ("patch"). Additionally, the present invention reveals the possibility of incorporating and to solubilize in a same hydrogel liposoluble or hydrosoluble vitamins.

Finally a process for the preparation of dietary complement and a dermatological crème incorporating the hydrogel of the present invention.

It is important to recognize that the terms "dermatology" and "dermatological" are used in a broad sense, therefore including all other cosmological and cosmetic applications. In addition, these terms extend themselves to applications on the skin or on the phanera.

The term "dietary complement" also extends itself in a broad sense, therefore including any dietary preparation, the compliment being used in a nutritional or therapeutic role or in a yet simple physical role in dietary preparations, for example, as a texturing agent, as a filling agent or as a viscosity controlling agent.

The compositions of the present invention are primarily destined for humans but may also find application in the veterinary field.

Referring to figure 1, one can note that the hydrogel of the present invention is a chitosane and xanthane based complex, resulting from an ionic reaction between these two polyions. A process for preparing the hydrogel is described in US patent 5,720,206 granted to the same assignee as the present invention. As illustrated in figure 1, a complex is formed between xanthane and chitosane, that is, the establishment of various ionic bonds between chitosane and xanthane molecules, so forming the hydrogel.

Incorporation of liposoluble vitamins in the chitosane-xanthane hydrogel

Vitamin A

Vitamin A (Retinol) is a very light and oxygen sensitive product. The use of this product, for example, in a crème can't be accomplished unless it is stabilized beforehand against the harmful effects of the agents previously cited. The process of the present invention consists in stabilizing vitamin A in a hydrogel composed of xanthane and chitosane, prepared according to the method described in US patent 5,620,706.

Example 1 inclusion of vitamin A during the preparation

A solution (100 ml) of vitamin A (10-20% w/v) in ethanol is first prepared. This solution is then added, under vigorous stirring, to a xanthane solution (500 ml) 0.65% w/v, to finally obtain a final concentration in vitamin A of 1.66 - 3.33% w/v. The solution can be preserved at 3 °C. A pulverization system is then employed in order to add the vitamin A – xanthane solution to a chitosane solution (0.65% w/v). The reaction has to be sustained for 30 min. The so-formed gel has to be filtered and washed with water in order to reach a pH of 6.8. To increase the final stability of the gel, a final washing with a sodium bicarbonate solution (1% w/v) is effected, which brings the gel to a pH of 7.5. The gel

is then frozen and lyophilized. All the operations, including the freezing, are carried out in the absence of oxygen and light.

Example 2 Inclusion of vitamin A by diffusion

It is possible to introduce vitamin A by diffusion, by using a xanthane-chitosane based hydrogel possessing a degree of swelling (α) of at least 2000%. The degree of swelling is defined according to the following equation:

$$\alpha = 100\,\% \times \frac{\text{weight of swelled hydrogel at equilibrium - weight of dry hydrogel}}{\text{weight of dry hydrogel}}$$

Under these conditions the operating times are significantly reduced, in the process preventing the degradation of the molecule. To accomplish this, it suffices to dissolve 0.07 g of vitamin A in 1 ml of ethanol (96%) and to add to this solution 1.5 g of the xanthane – chitosane complex lyophilized with (α) = 2500%. A light stirring allows for the achievement of a homogeneous paste. This is followed by the addition of ethanol (2 ml) and water (200 µl), while maintaining a light stirring. The whole is then placed at 4°C for 24 h shielded from light. The alcohol is then evaporated at 4 °C. The final product possesses a vitamin A concentration of 46 mg / g lyophilized product.

Example 3 Inclusion of vitamin E

For the incorporation of Vitamin E, the process (1) developed for vitamin A is applied. The concentration of vitamin E can reach up to 20%.

Example 4 Inclusion of vitamin K

As far as vitamin K is concerned, the same process (1) as for vitamin A is used. The concentration of vitamin K can reach up to 20%.

Incorporation of water soluble vitamins

For water-soluble bio-active products, it is preferable to use the diffusion process for the incorporation of these products into the lyophilized hydrogel, in order to avoid inevitable losses of products during the reaction between xanthane and chitosane. Under these conditions, it is essential to have a hydrogel with a degree of swelling of at least 2000%.

Example 5 Inclusion of vitamin C

Since vitamin C presents a strong redox potential with regards to chitosane, a novel process of inclusion was developed. This process comprises two (2) steps:

Step 1 The preparation of the xanthane – chitosane complex (CHITOSANE^{MC}), that is the polyionic hydrogel;

Step 2 The incorporation of vitamin C

1. Preparation of the xanthane – chitosane complex

The CHITOSANE^{MC} complex is prepared according to the method described earlier in US patent 5,620,706. The chitosane used in the preparation of this complex, typically has molecular weight ranging from 250,000 to 350,000, and the hydrogel has a degree of swelling (α) of $\geq$ 2000%.

The CHITOSANE^{MC} is ground in order to obtain a fine powder, with particles having a diameter ranging between 250 and 500 μm.

2. Incorporation of vitamin C

2a. Stabilization by amino acids

To 10 ml of water is added vitamin C (1 g), L-cysteine (0.06 g), L-cystine (0.02 g) and L-methionine (0.02 g). Lyophilized CHITOXANE^{MC} (1 g), having a particle size ranging from 250 - 500 μm, is

then added. The mixture cannot contain any excess liquid phase. If necessary, water can be added. The mixture is maintained for 2 hours in order to achieve the hydration equilibrium. All the operations have to be carried out in the absence of light. The mixture is then frozen and lyophilized. The transformation into a final powder like product, yields particles varying in size from 50 to 125 μm.

The so-incorporated hydrated vitamin C presents a stability without any color change of 2 weeks at 45 °C, and of 20 weeks for the dry gel at the same temperature. It is possible to use tartaric acid (0.1%), metaphosphoric acid (0.03%), or citric acid (0.1%) as a stabilizing agent. The weighted percentage is in relation to the CHITOXANE^{MC} complex.

2b. Stabilization by tripeptides

Method 2a is used, by replacing the amino acids by a tripeptide with sulfurized amino acids. To 10 ml of water is added vitamin C (1 g) and glutathione (0.002 g). After stirring for 5 minutes, CHITOXANE^{MC} (1 g) is introduced and the mixture lightly stirred until a homogenous paste is obtained. The mixture is maintained for 2 hours in order to arrive at the hydration equilibrium. The paste is then frozen and lyophilized. All the operations have to be carried out in the absence of light.

Extraction and dosage of vitamin C in CHITOXANE^{MC}

Extraction

Extraction solvent: aqueous solution of metaphosphoric acid (3% w/v) and acetic acid (8% v/v)

Process: In a 50 ml centrifugation tube protected against light, was added from about 20 to 30 mg of lyophilized CHITOXANE^{MC} + Vit. C and 40 ml of the extraction solvent. The whole was stirred for 60 minutes with a magnetic stirrer. The suspension was finally centrifuged (4000 rpm) and the supernatant analyzed.

Quantitative determination of vitamin C

Before proceeding at the quantitative determination of vitamin C, the maximum absorption has to be measured in a UV-VIS spectrophotometer. The standard is prepared by using a vitamin C solution, which served in the manufacture of CHITOXANE^{MC} - Vit. C, in the extraction solvent. The test revealed a maximum absorption at 243 nm for vitamin C of the Aldrich type, purity 98%.

After having evaluated the maximum absorption, the standard absorption curve is determined (absorption in function of concentration of vitamin C). A solution of vitamin C of a concentration of 1.3 mg/ml was prepared in a gauged flask with the extraction solvent. The solution is prepared just prior to the analysis procedure. The absorption / concentration (mg/ml) dependency was measured by successive dilutions.

The concentration of the supernatant obtained in the extraction is determined by photocolorimetry at 243 nm and is calculated from the standard curve.

The concentration of vitamin C in the sample prepared according to process 2a is 49.6%.

Inclusion of CHITOXANE^{MC}-Vit. C in a cream base

CHITOXANE^{MC}-Vit. C (1 g) is hydrated with water, until a cream like paste is obtained. It is then sufficient to weigh the paste and to calculate the obtained concentration in vitamin C. The paste is subsequently incorporated in the cream base while stirring vigorously, in order to obtain a final concentration in vitamin C, preferably about 5 to 25% by weight.

Determination of the stability of vitamin C included in a cream base.

The preparation obtained by inclusion of CHITOXANE^{MC}-Vit. C in a cream base (10 g) is introduced in a light protected tube. This tube is then heated at 45 °C. To determine the degree of degradation of vitamin

C in the cream base, a sample corresponding to about 10-30 mg of vitamin C was drawn. This sample was drawn every day for the first 4 days and then every second day for a total of 30 days. If the samples are not immediately analyzed, they have to be kept refrigerated (-4 °C).

The drawn samples are analyzed as described previously (always using the extraction solvent).

Creams having a concentration ranging from 5 to 25% by weight in vitamin C are prepared from samples VS2L, VS2R and VS2M. By using the described method, a very good stability for vitamin C included in CHITOXANE was found: no coloration of the cream after heating at 45 °C for 30 days while the decomposition of vitamin C was 15%. Under the same conditions, for a cream prepared from free vitamin C, a decomposition of 98% and an orange discoloration could be observed.

Kinetics of release

Referring to Table 1 below, three types of CHITOXANE^{MC}-Vit. C hydrogels were studied. As illustrated by figure 2, the release kinetics of vitamin C are determined by introducing a precise amount of CHITOXANE^{MC}-Vit. C in the reactor. The kinetic release curves were obtained at ambient temperature in the presence of light, by using a vitamin C stabilizer comprised of metaphosphoric acid (3% / vol) and acetic acid (8% / vol).

Table 1

Code for CHITOXANE^{MC}-Vit. C preparations	Degree of swelling (α) of CHITOXANE^{MC} (%)	Concentration of vitamin C in CHITOXANE^{MC}-Vit. C (%)
VS2L	1800	50.3
VS2R	2200	49.6
VS2M	3500	51.0

The solvent (a mixture composed of 3% w/v metaphosphoric acid and 8% w/v acetic acid) penetrates directly into the reactor via a central pipe, where it enters into contact with the CHITOXANE^{MC}-Vitamine C. The vitamin C is gradually released from the complex and dissolves in the solvent. The solvent is drained from the reactor via small apertures, into an exterior pipe. By using this method, a constant circulation of the solvent around the sample to be analyzed, is assured.

It can be observed, by reference to figure 3, that there exists a linear relationship between vitamin C released and time (zero order kinetics), with a clean break after 60 minutes for CHITOXANE^{MC}-Vit. C of type VS2L and with a clean break after 40 minutes for CHITOXANE^{MC}-Vit. C of type VS2R.

It can be observed, by reference to figure 4, that in a first case (CHITOXANE^{MC}-Vit. C of type VS2L), vitamin C diffuses at a constant rate of 0.36 mg/min over a first period, and that the rate decreases by half for the next 100 minutes.

In a second case, as can be observed in figures 5 and 6, the diffusion takes place at a considerably faster rate during the first 40 minutes with a rate of 1.18 mg/min, and is then almost non-existent with a rate of 0.02 mg/min.

Figure 5 illustrates the weight of vitamin C released in function of time for coded sample VS2R Hydrogel CHITOXANE^{MC}-Vit. C, prepared from CHITOXANE^{MC} possessing a degree of swelling (α) = 2000%. Figure 6 illustrates the release rate in function of time of vitamin C, for coded sample VS2R.

Figure 7 illustrates the variation in function of time of the percentage of released vitamin C for a sample VS2M Hydrogel CHITOXANE^{MC}-Vit. C, prepared from CHITOXANE^{MC} possessing a degree of swelling (α) = 3500%. A release in vitamin C of 85% is noted for this sample, during the first 10 minutes of elution.

Inclusion of CHITOXANE^{MC}-Vit. C in a dietary preparation

The CHITOXANE^{MC}-Vit. C hydrogel and other chitosane-xanthane hydrogels containing vitamins, nucleic acids, amino acids and oligopeptides, can also be used in hydrated dietary preparations such as gels, sauces and syrups as well as dehydrated preparations.

The physical characteristics of the selected chitosane-xanthane hydrogel, will determine the structure and the more or less viscous texture of the hydrogel. According to the present invention, the choice of hydrogel will therefore allow for the hydrogel to be adapted to various dietary applications.

In addition, the present invention comprises the formation of pellets from CHITOXANE^{MC}-Vit. C powder with other active ingredients, if required. The formation of pellets from the powder of other lyophilized chitosane-xanthane hydrogels containing various vitamins, amino acids, nucleic acids, oligopeptides or a combination selected from these active ingredients, is also provided for.

It is to be understood however, that various changes and modifications within the spirit and scope of the present application as described, that do not have a direct and material effect upon the way the

invention works will become apparent to those skilled in the art and are to be covered by the following claims.